PATENT

Attorney Docket No.: 050179-0080

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
)
Himanshu N. BRAHMBHATT, et al.)
) Group Art Unit: 1655
Serial No.: 09/530,772)
) Examiner: Chakrabarti, A.
Filed: June 30, 2000)
)
For: SUICIDE EXPRESSION VECTOR FOR USE)
IN VACCINE STRAINS)

PRELIMINARY AMENDMENT

Honorable Assistant Commissioner for Patents
Washington, DC 20231

Dear Sir:

Prior to examination of the above-captioned Request for Continued Examination (RCE) patent application, please amend the claims as set forth below and consider the arguments herein presented in response to the Advisory Action dated March 19, 2002.

IN THE CLAIMS

Please add Claims 20 and 21, as follow.

20. A method of transiently expressing a heterologous peptide, polypeptide or protein in a selected host cell, comprising;

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(i) providing a suicide vector according to any one of claims 1 to 12;

(ii) transforming said host cell with said suicide expression vector;

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03 FC:103 234.00 CH
04 FC:102 84.00 CH

(iii) culturing said transformed host cell under suitable conditions for the expression of the said heterologous peptide, polypeptide or protein, and

(iv) thereafter inducing expression of the restriction enzyme or functional portion thereof to bring about cleavage and subsequent degradation of the said suicide expression vector to thereby remove recombinant vector DNA from the host cell and halt further expression of the said heterologous peptide, polypeptide or protein.

21. A suicide expression vector for transiently expressing a heterologous peptide, polypeptide or protein in a selected host cell, said vector comprising;

(i) a first nucleotide sequence encoding said heterologous peptide, polypeptide or protein operably linked to a first promoter sequence,

(ii) a second nucleotide sequence encoding a restriction enzyme or functional portion thereof operably linked to a second promoter sequence, said second promoter sequence being inducible, and

(iii) one or more cleavage site(s) for said restriction enzyme or functional portion thereof, said cleavage site(s) being absent from the chromosomal DNA of said host cell, wherein upon introduction of the vector into said host cell, induced expression of the restriction enzyme or functional portion thereof from said second nucleotide sequence brings about the cleavage and subsequent degradation of the suicide expression vector to thereby remove recombinant vector DNA from the host cell and holt further expression of the said heterologous peptide, polypeptide or protein.

Please amend Claims 1, 6, 8-10, 15, 17 and 19, as follow.

1. (Amended) A suicide expression vector for transiently expressing a heterologous peptide, polypeptide or protein in a selected host cell, said vector comprising;

(i) a first nucleotide sequence encoding said heterologous peptide, polypeptide or protein operably linked to a first promoter sequence,

(ii) a second nucleotide sequence encoding a restriction enzyme or functional portion thereof operably linked to a second promoter sequence, said second promoter sequence being inducible, and

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(iii) one or more cleavage site(s) for said restriction enzyme or functional portion thereof, said cleavage site(s) being absent from the chromosomal DNA of said host cell, wherein upon introduction of the vector into said host cell, induced expression of the restriction enzyme or functional portion thereof from said second nucleotide sequence brings about the cleavage and subsequent degradation of the suicide expression vector.

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6. (Twice Amended) The vector according to claim 1, wherein the second nucleotide sequence encodes a restriction enzyme or functional portion thereof that recognizes a cleavage site(s) of ten or more nucleotides.

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8. (Twice Amended) The vector according to claim 1, wherein the one or more cleavage site(s) is/are located at a site(s) on the vector which avoids steric hindrance of binding by said restriction enzyme or functional portion thereof.

D4

9. (Twice Amended) The vector according to claim 1, further comprising a third nucleotide sequence encoding a ribozyme targeted against mRNA produced from the said second nucleotide sequence encoding the restriction enzyme or functional portion thereof.

D4

10. (Twice Amended) The vector according to claim 1, wherein the second promoter is selected from the group consisting of the placZ promoter, the placUV5 promoter and the T7 RNA polymerase promoter.

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15. (Amended) A method of transiently expressing a heterologous peptide, polypeptide or protein in a selected host cell, comprising;

(i) providing a suicide vector according to any one of claims 1 to 12;

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(ii) transforming said host cell with said suicide expression vector;

(iii) culturing said transformed host cell under suitable conditions for the expression of the said heterologous peptide, polypeptide or protein, and

(iv) thereafter inducing expression of the restriction enzyme or functional portion thereof to bring about cleavage and subsequent degradation of the said suicide expression vector, thereby transiently expressing said heterologous peptide, polypeptide or protein.

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17. (Amended) A method for the production of a microorganism vector which contains recombinant peptide, polypeptide or protein but no recombinant DNA, comprising;

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(i) providing a suicide expression vector according to any one of claims 1 to 12;

(ii) transforming said microorganism with said suicide expression vector;

(iii) culturing said transformed microorganism under suitable conditions for the expression of the said heterologous peptide, polypeptide or protein, and

(iv) thereafter inducing expression of the restriction enzyme or functional portion thereof to bring about cleavage and subsequent degradation of the said suicide expression vector, thereby producing said microorganism vector free from recombinant DNA.

D6

19. (Amended) A microorganism vector produced by the method according to claim 17.

D7

REMARKS

Claims 1-19 are pending in the application. Claims 20 and 21 are added and Claims 1, 6, 8-10, 15, 17 and 19 are amended in an effort to encompass inventive subject matter. A Declaration Under 37 C.F.R. 1.132 is herein submitted. No new matter is added into the application.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned, "Version With Markings To Show Changes Made."

Objections

Claims 8 through 12 stand objected to for being in improper form because a multiple dependent claim cannot depend on another multiple dependent claim. Applicants herein amend Claims 6 and 8 through 10 to correct dependency from multiple dependent claims. Accordingly, Applicants respectfully request reconsideration and withdrawal of this objection.

Rejections Under 35 USC §112

Claims 15 through 19 stand rejected as being indefinite under 35 USC §112 because the instantly claimed methods lack a final process step that clearly relates back to the preamble. Applicants respectfully traverse this rejection for at least the following reasons.

Applicants submit that the claims as herein presented are not indefinite. In particular, the phrase "and subsequent degradation" is added to Claims 15 and 17 to more clearly describe the intended steps of the method to be submitted. In addition, the insertion of ", thereby transiently expressing said heterologous peptide, polypeptide or protein" to Claim 15, and ", thereby producing said microorganism vector free from recombinant DNA" to Claim 17, clearly states the accomplishments of each method and also relates back to the method stated in the respective preamble. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 USC §112.

Rejections Under 35 USC §102

Claims 1, 2, 6 and 13 through 19 stand rejected under 35 USC §102(b) over Herrero et al. (Journal of Bacteriology, (1990), Vol. 172, No. 11, pages 6557-6567) as of the final Office Action dated September 6, 2001. This rejection is respectfully traversed for at least the following reasons.

Applicants respectfully traverse the Office Actions conclusion that Herrero et al. anticipates the claimed invention. Applicants submit that Herrero et al does not disclose each and every element of Applicant's invention as herein claimed. In particular, Herrero et al. do not disclose an expression vector comprising in relevant part, a first nucleotide encoding a heterologous peptide, a second nucleotide encoding a restriction enzyme, and cleavage sites for

said restriction enzyme, said cleavage sites being absent from the chromosomal DNA of the host cell, wherein induced expression of the restriction enzyme brings about cleavage and subsequent degradation of the expression vector to thereby transiently express the heterologous.

As noted by the Examiner at pages 13 through 14 of the final Office Action, Applicants respectfully submit that the present invention is distinguished over the prior art as the present invention allows (i) expression of a suitable amount of the heterologous peptide encoded by a first nucleotide sequence to accumulate in the host cell, and (ii) the expression of a restriction enzyme which brings about the cleavage and subsequent degradation of the suicide expression vector in the host cell. The present invention thereby allows for the complete removal of the suicide expression vector from the host cell. Claims 1, 15 and 17 as herein presented more clearly set forth the transient expression of the heterologous peptide, polypeptide or protein, and the subsequent degradation of the expression vector.

Support for the subsequent degradation of the expression vector is on page 2, lines 17-23 of the specification, wherein it is explained that the suicide expression vector of the present invention may be transformed into a host cell and used to produce a desired heterologous peptide, polypeptide or protein. More specifically, the specification states, "Once sufficient expression of the peptide, polypeptide or protein has occurred, the transformed host cell may be induced to express the restriction enzyme or functional portion thereof thereby causing the cleavage and subsequent degradation of the expression vector."

In contrast, Herrero et al. discloses a transposase to integrate genes "into the chromosome of target bacteria to generate stable, non-antibiotic-resistant hybrids." (Herrero et al., at 6565). Importantly Applicants submit that the transposon in Herrero et al. is transposed and not degraded. That is, following transposition the host target DNA comprises host DNA plus the

transposon. Accordingly, it is submitted, the transposon does not allow for the complete degradation of the expression vector and transient expression of the desired peptide.

In addition, unlike Applicants invention, Herrero et al. do not disclose a second nucleotide encoding a restriction enzyme, which expression thereof results in the cleavage of the recombinant expression vector only. Moreover, the instant specification specifically sets forth on page 2 that the restriction enzyme is carefully selected "so as to ensure that the expression of the restriction enzyme brings about the cleavage of the recombinant expression vector only" and further, "the host's DNA is not cleaved by the expressed restriction enzyme."

In contrast, Herrero et al. teaches the use of transposases to splice and ligate the transposon (tn5 or Tn10) from donor DNA into a target plasmid or into the chromosome of target bacteria. Applicants submit that transposases cleave two sites (inverted terminal repeats) on the original DNA and another random site on the host DNA sequence. Furthermore, unlike a restriction enzyme, Applicants submit that a transposase has recombinase activity. Applicants respectfully submit, therefore, in view of the claimed invention, a person skilled in the art would not consider that a transposase is a restriction enzyme and attach hereto a Declaration Under 37 C.F.R. 1.132 by one of the inventors, Robert Seymour, setting forth the different enzymatic activities and functions of a transposase and restriction enzyme.

Applicants respectfully request reconsideration and withdrawal of the rejections under 35 USC §102(b).

Rejections Under 35 USC §103

Claims 1, 2, 6, 7 and 13-19 are rejected under 35 USC §103(a) over Herrero et al. (Journal of Bacteriology, (1990), Vol. 172, No. 11, pages 6557-6567) in view of Marshall et al.

(U.S. Patent 5,420,032) (May 30, 1995) as of the final Office Action dated September 6, 2001. Applicants respectfully traverse this rejection for at least the following reasons.

Herrero et al. in view of Marshall et al. do not teach the suicide expression vector of Applicants' invention. For the reasons stated above, Herrero et al. do not disclose the claimed suicide expression vector comprising, in relevant part, a first nucleotide encoding a heterologous peptide, a second nucleotide encoding a restriction enzyme, and cleavage sites for said restriction enzyme, said cleavage sites being absent from the chromosomal DNA of the host cell, wherein induced expression of the restriction enzyme brings about cleavage and subsequent degradation of the expression vector to thereby transiently express the heterologous peptide. Further, Marshall et al. do not cure the deficiencies of Herrero et al. Although Marshall et al. describes a homing endonuclease named I-CeuI, using I-CeuI in combination with Herrero et al. does not teach that the restriction enzyme is absent from the chromosomal DNA nor do the combined references teach that the restriction enzyme brings about the cleavage and subsequent degradation of the suicide expression vector without affecting the host DNA. In addition, the suicide capabilities (*pir* gene) of the plasmid disclosed in Herrero et al, do not relate to the presence or activity of the transposase on the plasmid. As a result, substitution of the restriction enzyme described in Marshall et al., does not teach any closer to the present invention.

Finally, neither Herrero et al., nor Marshall et al., allow for the complete removal of the heterogenous DNA from the cell. For these reasons it is submitted that Herrero et al., and Marshall et al., do not teach nor disclose the present invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejections over Herrero et al. in view of Marshall et al.

Claims 1, 2, 6, 7 and 13 through 19 also stand rejected under 35 USC §103(a) over Herrero et al. in view of Hardy et al. (U.S. Patent No. 5,851,817); Herrero et al. in view of Calvet et al.; and Herrero et al. in view of Kemp et al. as of the final Office Action dated September 6, 2001. Applicants respectfully traverse these rejections for at least the following reasons.

Herrero et al. in view of Hardy et al.; Herrero et al. in view of Calvet et al. (U.S. Patent No. 5,552,313); and Herrero et al. in view of Kemp et al. (U.S. Patent No. 6,111,070) do not teach the suicide expression vector of Applicants' invention. For the reasons stated above, Herrero et al. do not disclose the claimed suicide expression vector and not one of Hardy et al., Calvet et al., or Kemp et al. cure the deficiencies of Herrero et al. Applicants submit that Hardy et al., Calvet et al., and Kemp et al. do not disclose a suicide plasmid that utilizes inducible expression of a restriction enzyme to cleave the vector and cause its degradation. With respect to Claim 3 of the claimed invention, Hardy et al., merely describe a vector that expresses a contraceptive antigen, however this is in no way a "suicide vector" that subsequently degrades itself without affecting the host DNA. Likewise, with respect to Claim 4 of the claimed invention, Calvet et al., merely describe a vector that expresses an esterase capable of hydrolyzing organophosphates, however this is in no way a "suicide vector" that subsequently degrades itself without affecting the host DNA. Lastly, with respect to Claim 5 of the claimed invention, Kemp et al. merely describe a vector that expresses an insecticidal toxin, however again, this is in no way a "suicide vector" that subsequently degrades itself without affecting the host DNA. Applicants submit that Herrero et al. in view of Hardy et al., Calvet et al., or Kemp et al. do not teach an expression vector comprising, in relevant part, the second nucleotide sequence bringing about cleavage and subsequent degradation of the suicide expression vector such that the suicide expression vector is removed and therefore the heterologous peptide, polypeptide or

protein is no longer expressed in the host cell. Consequently, the rejections should be reconsidered and withdrawn and such favorable action is respectfully requested.

Claims 1, 2, 6, 7, 9 and 13 through 19 stand rejected under 35 USC 103(a) over Herrero et al. in view of Barber et al. (U.S. Patent No. 6,043,077)) as of the final Office Action dated September 6, 2001. Applicants respectfully traverse this rejection for at least the following reasons.

Herrero et al. in view of Barber et al. do not teach the suicide expression vector of Applicants' invention wherein a nucleotide sequence encodes a ribosome targeted at specific mRNA. For the reasons stated above, Herrero et al. do not disclose the claimed suicide expression vector and Barber et al. do not cure the deficiencies of Herrero et al. Applicants submit that neither Herrero et al. nor Barber et al. teach an expression vector comprising, in relevant part, the second nucleotide sequence bringing about cleavage and subsequent degradation of the suicide expression vector such that the suicide expression vector is removed and therefore the heterologous peptide, polypeptide or protein is no longer expressed in the host cell.

Furthermore, with reference to Claim 9, Barber et al. disclose a method wherein rRNA is used to target a disease rRNA, for example ribosomal RNA is used for targeting an oncogene. In contrast, Applicants' invention discloses that the third nucleotide sequence encodes ribosomal rRNA to control leaky expression of the second promoter sequence. More specifically, page 4 of the claimed invention states, "As a "safe guard" against premature expression of the restriction enzyme or functional portion thereof, the expression vector preferably comprises a nucleotide sequence encoding a ribosome targeted against the mRNA produced from the nucleotide sequence encoding the restriction enzyme or functional portion thereof. The ribosome should be

expressed such that it will be present to immediately cleave low "leakage" amounts of mRNA encoding the restriction enzyme of functional portion thereof." Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

Conclusion

If any issues remain outstanding or if an Examiner's amendment could be made to expedite prosecution, then Applicants respectfully invite the Examiner to contact the undersigned representative at the telephone number listed below.

Please grant any extensions of time deemed necessary for entry of this communication. Please charge any deficient fees, including Notice of Appeal fees, or credit any overpayment of fees, to Deposit Account No. 5000417.

Respectfully submitted,

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